

DO MICROBES REDUCE PHOSPHATE?

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Do Microbes Reduce Phosphate?

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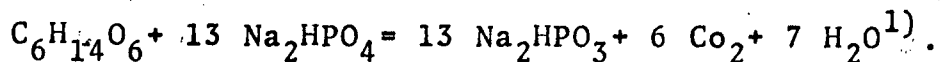
by F. Liebert

On page 202 of volume 70 of this publication we find a paper by K. S. Rudakov from the bacteriological agronomical station in Moscow, in which he draws the conclusion that optional anacrobic bacteria are capable of reducing phosphate, a process, wherein the phosphate assumes the role of the sodium nitrate during denitrification. In the course of this reduction there was said to form the extremely poisonous phosphoric acid, subphosphoric acid and even PH_3 . (Rudakov always writes "phosphorous" acid, but probably means to say subphosphoric acid H_3PO_2 .)

It needs no particular emphasis that if K. S. Rudakov is proven right, he would have discovered a particularly interesting microbiological process of greatest importance for agriculture and fisheries which for instance could offer an explanation for otherwise unsolvable incidences of dying of fish. So I allow myself to offer the following comments to the above mentioned paper.

If we raise the question: "Do microbes reduce phosphates under the same circumstances in which nitrate acts during denitrification?" then it immediately results that the reduction of phosphate supplies energy to the bacteria here as well as during denitrification. Thermochemistry makes it

possible to compute the amount of calories which here during reduction of phosphate and oxidation of mannite are liberated or are being consumed. If we now offer to the bacteria mannite as a source of carbon and the neutrally reacting Na_2HPO_4 as a source of phosphate, and then further assume that the phosphate be reduced to phosphite, then we can express this reaction stoichiometrically:



Mannite

Bisodium Phosphite

With the assistance of the heat of formation of the molecules present in this equation and the law of Hess (1840) it is now easy to calculate whether this reaction supplies energy or absorbs it.

Since the tables, for instance Landolt-Börnstein, "physical-chemical tables", generally do not give values for the heat of formation of organic compounds, but instead give values for the direct experimentally determined heat of combustion, then we are forced to use it to calculate the heat of formation of mannite. Heat of formation of $\text{C}_6\text{H}_{14}\text{O}_6$ = heat of formation of 6 CO_2 and 7 H_2O reduced by the heat of combustion of $\text{C}_6\text{H}_{14}\text{O}_6$, that is $6 \times 94.34 + 7 \times 68.3 - 728 = 316$ kg. calories per gram mol mannite. The energy balance can be calculated as follows:

Heat of formation of 1 gram mol mannite = 316

" " " " 13 " Na_2HPO_4 = 5390

Total 5706 kg. cal.

1) In a kinetic sense such a reaction is of course senseless.

Heat of formation of 13 gram mol Na_2HPO_3 = 4460

" " " " 6 " CO_2 = 566

" " " " 7 " H_2O = 478

Total 5504 kg. cal.

If then 1 gram mol $\text{C}_6\text{H}_{14}\text{O}_6$ and 13 gram mol Na_2HPO_4 form out of the elements then 5706 kg. calories are liberated, if however, the equal amount of elements form 13 gram mol Na_2HPO_3 , 6 gram mol CO_2 and 7 gram mol H_2O only 5504 kg. calories are liberated.

The reduction of phosphate to phosphite therefore does not supply the bacteria with energy, as Rudakov indicates, but instead consumes energy.

What happens now with the reduction to hypophosphite which can be expressed by the equation: $2 \text{C}_6\text{H}_{14}\text{O}_6 + 13 \text{Na}_2\text{HPO}_4 = 13 \text{NaH}_2\text{PO}_2 + 11 \text{NaHCO}_3 + \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}$ since the subphosphorous acid is mono-basic?

In the same manner as above it can be calculated that this reduction of phosphate consumes 483 kg. calories.

And finally we can imagine the reduction of phosphate to have progressed to PH_3 which can be expressed: $4 \text{C}_6\text{H}_{14}\text{O}_6 + 13 \text{Na}_2\text{HPO}_4 = 13 \text{PH}_3 + 22 \text{NaHCO}_3 + 2 \text{CO}_2 + 4 \text{H}_2\text{O}$ and whereby we calculate that this reduction requires no less than 1147 kg. calories!

We have now found out that the reduction to Na_2HPO_3 , NaH_2PO_2 or PH_3 yields the microbes no gain in energy.

What is the situation at the denitrification, the re-

action of which can be written as follows: $5 \text{ C}_6\text{H}_{14}\text{O}_6 + 26 \text{ KNO}_3 = 26 \text{ KHCO}_3 + 4 \text{ CO}_2 + 22 \text{ H}_2\text{O} + 13 \text{ N}_2$? This equation has as a result that here the reduction of sodium nitrate yields no less than 3270 kg. calories.

For better comparison the above mentioned values have been converted to 1 gram mol of mannite in the table below:

Energy gain by reduction to Na_2HPO_3	- 202 kg. cal.
" " " " " NaH_2PO_2	- 241 kg. "
" " " " " PH_3	- 286 kg. "
" " " denitrification	+ 654 kg. "

These figures demonstrate convincingly, that even if a reduction of phosphates is taking place through the activity of microbes, this process never proceeds analog to the one of denitrification since no energy is being supplied to the bacteria which the reduction of nitrates does to a large degree.

It shall also be commented here that above we manipulated with sodium phosphates; whether we consider ammonium salts or sour phosphates has no bearing on the result.

If the oxidation of mannite were accompanied not only by the formation of CO_2 and H_2O but for instance acids as well, then even more energy would be required to reduce the phosphates.

If we above proved the opinion of Rudakov, that the reduction of phosphate supplies energy to the bacteria, to be completely false, then it follows immediately that its nutrient solution i.e. 2% mannite, 0.1-0.2% $\text{NH}_4\text{H}_2\text{PO}_4$, 15% CaCO_3 in tap water, does not represent a particularly select-

ive culture since whatever microbes accumulate in this environment, they would still have to extract their energy from a different source than the phosphate reduction. If we raise the question where this source may be then it becomes immediately clear that here with anacrobic cultivation only the fermentation of the mannite is able to supply energy to the microbes. To prevent any misunderstanding, I would like to comment right now that the above statements are not meant to assert that a phosphate reduction is not at all possible. But one is entitled to the conclusion that it is highly improbable that such large amounts of phosphate in such a short time could have been reduced as Rudakov thinks to have experienced. Formation of PH_3 , H_3PO_3 and H_3PO_2 is not a fuel reversible process and one can only expect to find very small quantities of reduction products of phosphorous acid.

For Rudakov to say, that in the culture medium fluids used by him the bacteria, with exception of the phosphate, were not offered any easily reducible substances which could offer them oxygen ignores the presence of the mannite; at fermentation this is a source of carbohydrates as well as oxygen.

It is not without interest to note, that the phosphate reduction costs far more energy than the reduction of carbon dioxide to carbon monoxide by means of mannite, because the reaction $\text{C}_6\text{H}_{14}\text{O}_6 + 7 \text{CO}_2 = 13 \text{CO} + 7 \text{H}_2\text{O}$ requires only 141 kg. calories per gram mol mannite, the reduction of phosphate

to phosphite however requires 202 kg. calories. Carbon dioxide therefore would, according to Rudakov, constitute therefore a far better source of oxygen for the bacteria than would phosphate and one would be more likely to encounter in the cultures of Rudakov CO instead of H_3PO_3 ! I should like to inject here that CO, as far as I am aware, has never been found as a product of microbe activity.

As could be expected, I always obtained very good amounts of coli and butyric acid ferments accompanied by strong formation of carbon dioxide while performing 25 experiments with garden or pasture soil and Rudakov's culture solution. The fermentation can of course be easier observed in stoppered bottles per Beijerinck than in test tubes with pyrogalllic alkali, but even here it could be plainly observed at all times.

Curiously, the Russian paper makes no mention at all of the fermentation, only the note to the effect that meatagar had been torn provides a hint that Rudakov's culture also fermented.

That Rudakov's culture fluid, which he portioned towards phosphate reduction, yielded just the amounts of coli and butyric acid bacteria, could be verified even more accurately by my own bacilli cultures. The daubing of ordinary meat gelatine resulted practically in only pure cultures of typical coli. The butyric acid ferments were isolated anaerobically on malt extract agar.

Even if I considered these culture fluids to be very

select for the purpose of coli and butyric acid ferments, that does not mean that all strains of these bacteria groups are resigned to what is here offered them, since it is known that the here available oxygen source is not agreeable to all forms of these bacteria.

It shall be further noted, that the liquid in the cultures soon experiences an acid reaction, in disregard of the CaCO_3 lying at the bottom, in other words, the liquid is not in equilibrium with the precipitates. After 2-3 days, the pH was \pm 4.6, that is very acid, which reinforces strongly the selectivity of the accumulations.

Evidently one can rid the butyric acid bacteria very easily with these accumulations; to do that one only has to overinoculate before the spores are quite mature and therefore have become less sensitive to oxygen. In my opinion the bacilli culture, which Rudakov isolated, is in all probability only ordinary coli bacteria, which concurs with his description.

Since in my experiments always the rapid growing microbes accumulated, coli and butyric acid ferments for instance, I fail to understand, why, when inoculated with Russian soil, these common microbes should be expelled by the rare, phosphate reducing, optional anaerobic bacteria of Rudakov, which also reduce sulphate (sic.).

Of course the question now arises, how to interpret the results of the Rudakov analysis. Unfortunately he doesn't make a single statement how he solved the difficult problem to

establish the presence of phosphate in the presence of H_3PO_3 , H_3PO_2 , SiO_2 , Ca, Al, mannite and metabolic products. This is all the more regrettable as he believes himself to be in possession of a method which permits him to determine with accuracy the presence of P_2O_5 in only 10 ccm. of fluid to one hundredth of a milligram under the most difficult circumstances!

I have convinced myself by means of experiments, that the phosphate determinations, be it according to the molybdenum-, magnesium- or uranyl method, leave us in the lurch in the presence of H_3PO_3 .

Are now the analyses of Rudakov proof of the reduction of phosphate? Of course not, they only prove that with the unknown method of analysis more phosphate could be found in the control samples, than could be found in the inoculated samples. (Whether the entire phosphate could be recaptured cannot be ascertained in his figures therefore depriving us of this control.) The proof is therefore lacking, that in the final cultures disturbing effects like divergent reaction, formation of phosphorous acid ester, insoluble calcium phosphates etc. did not simulate the alleged disappearance of the phosphate.

In particular it would be advisable to use caution, since Rudakov nowhere makes mention of the double reaction of $\text{NH}_4\text{H}_2\text{PO}_4$ (which reacts very acid) with the precipitate CaCO_3 . This process develops quite differently in the sterile control samples as in the cultures and depends on

several circumstances, like liquid reaction, size of grain and quality of the CaCO_3 , increase of the Ca-ions through the formation of acid, escape of the reaction products etc. Since these conditions are completely different in the control samples from those which influence the precipitation in the cultures, then we cannot draw any conclusions from the phosphate becoming insoluble.

The lack of data regarding the method of analysis used makes it of course impossible to say with certainty, where Rudakov's error lies, but considering everything I am of the opinion that most likely the formation of insoluble calcium phosphates, which occurs less frequently in the control samples than it does in the inoculated cultures, has chiefly led him astray.¹⁾ If one assumes that my supposition really applies, then it can be easily explained, why an increase in phosphorous acid was noticeable when he "oxidated" using powerful lye. The strong solution, when heated, acts on insoluble calcium phosphates and brings them into solution to a large extent forming Ca(OH)_2 and K_3PO_4 . Incidentally it shall be noted that boiling with strong caustic potash attacks the glass noticeably which interferes with the phosphate determination; only if the lye is so concentrated that solid KOH is available, only then can phosphite be separated with formation of phosphate and oxygen ²⁾; of course the lye also smoothly extracts the phosphorous acid from the bacteria.

1) One compare page 205, Rudakov.

2) Treadwell, K., Lohrb. d. anal. Chemie. Loipzig 1914. p. 356.

Regarding the PH_3 , which Rudakov obtained from cultures, I have to note, that he lacks proof, since he directed the captured PH_3 immediately into bromine, that a volatile phosphorous acid ester did not simulate the formation of PH_3 . It is also remarkable that he does not state to have noticed the odor of PH_3 , which after all is exceptionally strong and characteristic.

Since the analysis of phosphorous acid in cultures is relatively laborious, I have embarked in a different direction to prove the formation of its reduction products. I thereby came to the conclusion, that H_3PO_3 as well as H_3PO_2 and PH_3 even with a very acid reaction reduce AgNO_3 at boiling heat, while mannite and the generated metabolic products and bacteria bodies do not.

If then one adds to the fermented culture liquid about half of its volume in 2 normal H_2SO_4 and an excess of AgNO_3 , then even a small content of reduction products will cause a brown to black discoloration of the phosphorous acid at boiling, which has its origin in the reduction of AgNO_3 . Obviously the discoloration offers no absolute proof of the presence of the looked for products, for instance, when the cultures have formed H_2S , but not having made its appearance one can say with great probability that it has not been formed.

In more than fifty experiments however, I have yet to achieve a discoloration in the fashion mentioned above.

To provide the best opportunity for the reduction, I have even started cultures in strong, tightly closed bottles, that is under increased pressure but even in this case not a single positive reaction on the reduction products of the phosphorous acid was obtained. I also paid particular attention to the odor of the cultures but never became aware of the particularly noticeable odor of PH_3 . Experiments, to indicate PH_3 in the generated fermentation gases by directing it through AgNO_3 solution also ended in a negative result; I had just as little success in only approximating the presence of the looked for reduction products analog to the method of Marsh (with $\text{Zn} + \text{H}_2\text{SO}_4$).

What additionally counts strongly against the formation of reduction products of phosphorous acid by means of microbes, is the fact that never have these poisonous products been noticed in the intestines of carnovora, where considerable amounts of phosphates are present, even though in the feces anaerobic conditions predominate.

At last I would like to comment, that as a result of my critique of the Rudakov paper one need not fear a dying of fish following heavy fertilization with phosphates.

Conclusion

1. It was derived from thermo-chemical data that phosphate reduction through mannite can never provide the bacteria with a gain in energy and it was further proven, again contrary to Rudakov's opinion, that this process, when it takes place, does not proceed analog to the process of

denitrification. --2. It was also shown, that the results of Rudakov's analyses do not by themselves constitute proof of a phosphate reduction. --3. Rudakov's culture solution in my hands always yielded an accumulation of coli and butyric acid ferments, as could be expected. --4. By means of several distinct qualitative reactions reduction products of the phosphorous acid were searched for, however, were never encountered.